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# Effects of the BET-inhibitor, RVX-208 on the HDL lipidome and glucose metabolism in individuals with prediabetes: A randomized controlled trial☆

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ARTICLE INFO

*Article history:*
Received 22 October 2015
Accepted 3 March 2016

ABSTRACT

*Aims.* High-density lipoprotein (HDL) and apolipoprotein A-I (apoA-I) can modulate glucose metabolism through multiple mechanisms. This study determined the effects of a novel bromodomain and extra-terminal (BET) inhibitor (RVX-208) and putative apoA-I inducer on lipid species contained within HDL (HDL lipidome) and glucose metabolism.

*Materials and methods.* Twenty unmedicated males with prediabetes received 100 mg b.i.d. RVX-208 and placebo for 29–33 days separated by a wash-out period in a randomized,

*Abbreviations:* ALT, alanine aminotransferase; AMPK, AMP-activated protein kinase; ApoA-I, apolipoprotein A-I; ApoA-II, apolipoprotein A-II; AST, aspartate aminotransferase; BET, bromodomain and extra-terminal; BH, Benjamini-Hochberg; BMI, body mass index; CE, cholesteryl ester; Cer, ceramide; CETP, cholesteryl ester transfer protein; COH, free cholesterol; DG, diacylglycerol; DHC, dihexosylceramide; dhCer, dihydroceramide; DI, disposition index; eGFR, estimated glomerular filtration rate; FBE, full blood examination; GGT, gamma-glutamyl transpeptidase; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; GM3, GM3 ganglioside; $HbA_{1c}$, glycated hemoglobin; HDL, high-density lipoprotein; HDL-C, HDL-cholesterol; hsCRP, high-sensitivity C-reactive protein; IDL, intermediate-density lipoprotein; LCAT, lecithin-cholesterol acyltransferase; LC-ESI-MS/MS, liquid chromatography, electrospray ionization tandem mass spectrometry; LDL, low-density lipoprotein; LDL-C, LDL-cholesterol; LFT, liver function test; LPC, lysophosphatidylcholine; LPC(O), lysoalkylphosphatidylcholine; LPE, lysophosphatidylethanolamine; LPI, lysophosphatidylinositol; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscle volume; MHC, monohexosylceramide; NMR, nuclear magnetic resonance; OGTT, oral glucose tolerance test; PC, phosphatidylcholine; PC(O), alkylphosphatidylcholine; PC(P), alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE(O), alkylphosphatidylethanolamine; PE(P), alkenylphosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; $R_a$, rate of appearance; $R_d$, rate of disappearance; RDW, red cell distribution width; rHDL, reconstituted HDL; S1P, sphingosine-1-phosphate; $S_I$, insulin sensitivity index; SM, sphingomyelin; T2DM, type 2 diabetes mellitus; TG, triglycerides/triacylglycerides; THC, trihexosylceramide; U&E, urea and electrolytes; VLDL, very low-density lipoprotein.

☆ ClinicalTrials.gov identifier: NCT01728467.

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http://dx.doi.org/10.1016/j.metabol.2016.03.002

*Keywords:*
Clinical trial
Glucose metabolism
Type 2 diabetes
High-density lipoprotein
Prediabetes

cross-over design trial. Plasma HDL-cholesterol and apoA-I were assessed as well as lipoprotein particle size and distribution using NMR spectroscopy. An oral glucose tolerance test (OGTT) protocol with oral and infused stable isotope tracers was employed to assess postprandial plasma glucose, indices of insulin secretion and insulin sensitivity, glucose kinetics and lipolysis. Whole plasma and HDL lipid profiles were measured using mass spectrometry.

*Results*. RVX-208 treatment for 4 weeks increased 6 sphingolipid and 4 phospholipid classes in the HDL lipidome ($p \leq 0.05$ versus placebo), but did not change conventional clinical lipid measures. The concentration of medium-sized HDL particles increased by 11% ($P = 0.01$) and small-sized HDL particles decreased by 10% ($P = 0.04$) after RVX-208 treatment. In response to a glucose load, after RVX-208 treatment, plasma glucose peaked at a similar level to placebo, but 30 min later with a more sustained elevation (treatment effect, $P = 0.003$). There was a reduction and delay in total ($P = 0.001$) and oral ($P = 0.003$) glucose rates of appearance in plasma and suppression of endogenous glucose production ($P = 0.014$) after RVX-208 treatment. The rate of glucose disappearance was also lower following RVX-208 ($P = 0.016$), with no effect on glucose oxidation or total glucose disposal.

*Conclusions*. RVX-208 increased 10 lipid classes in the plasma HDL fraction, without altering the concentrations of either apoA-I or HDL-cholesterol (HDL-C). RVX-208 delayed and reduced oral glucose absorption and endogenous glucose production, with plasma glucose maintained via reduced peripheral glucose disposal. If sustained, these effects may protect against the development of type 2 diabetes.



## 1. Introduction

HDL and its major apolipoprotein, apoA-I can directly modulate glucose metabolism through multiple mechanisms [1–3]. In the clinical setting, acute HDL elevation via short-term reconstituted HDL (rHDL) infusion [1] and chronically raising HDL via a cholesteryl ester transfer protein (CETP) inhibitor [3] reduce blood glucose in individuals with type 2 diabetes mellitus (T2DM). This is underpinned by at least two known mechanisms, namely increased insulin secretion [1,4,5] and enhanced skeletal muscle glucose uptake via an AMP-activated protein kinase (AMPK)-mediated mechanism [1]. HDL may also act via a third mechanism to increase insulin sensitivity via lipid removal and anti-inflammatory actions in metabolic tissues [6]. These newly described roles of HDL relating to glucose metabolism [2] suggest therapies that target HDL and/or apoA-I may have relevance in the management of T2DM.

The focus of HDL therapies has evolved in the light of recent data showing that HDL particle number, composition and function relate more closely to cardiovascular outcome than standard clinical measures of HDL-C content [7–9]. As a result, compounds targeting apoA-I are of particular interest due to their potential to increase HDL particle number and favorably alter the lipid composition and function of existing particles [8,10]. RVX-208 is an orally active small molecule which induces apoA-I through selective inhibition of BET proteins [11–13]. In African green monkeys, 60 mg/kg RVX-208 increased plasma apoA-I and HDL-C by 53% and 97% respectively after 28 days treatment [11]. In a follow-up phase I clinical trial in healthy volunteers RVX-208 (1–20 mg/kg/day) treatment for 7 days induced a 10% increase in plasma apoA-I and an 11% increase in the cholesterol efflux capacity of post-treatment plasma. To minimize potential liver transaminase elevations in humans, lower doses (100–150 mg b.i.d) of RVX-208 inducing more modest elevations in plasma apoA-I levels (3–6%) and HDL-C (6–8%) over a 12 week period have been investigated, with no evidence of hepatotoxicity [14]. Beyond the effects of RVX-208 on clinical lipid parameters, modification of the relative quantity of the hundreds of lipid species within HDL is one possible mechanism mediating the functional properties of HDL.

The objectives of the current study were to determine the effects of RVX-208 on the HDL lipidome and postprandial glucose metabolism in individuals with prediabetes following a glucose load. Effects on glucose kinetics, insulin secretion and whole-body insulin sensitivity were determined during a modified frequently sampled oral glucose tolerance test (OGTT) [15], incorporating stable isotope tracers.

## 2. Material and Methods

### 2.1. Patient Population and Screening

Twenty males aged 38–69 years with prediabetes based on WHO criteria (fasting blood glucose 6.1–6.9 mmol/L and/or 2 h blood glucose 7.8–11.0 mmol/L after a 75 g oral glucose load), body mass index (BMI) of 25–40 kg/m$^2$ and HDL-C levels ≤1.4 mmol/L were enrolled. Smoking, previous history of major illness and any prescription or over-the-counter medications were all exclusion criteria (see Supplementary Fig. 1 for CONSORT diagram). Patient characteristics and demographics are presented in Table 1. The study was approved by the Alfred Hospital Ethics Committee, performed in accordance with the Declaration of Helsinki (2008) and written informed consent was obtained from all participants.

For all visits, volunteers were required to attend The Alfred Hospital in the morning, following an overnight fast as well as having abstained from alcohol and caffeine for 24 h. At visit 1